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Appendix E1

Supplementary Methods

Clinical Scoring

We performed clinical assessment as follows: 0 = normal; 1 = flaccid tail; 2 = unable to use either hind leg; 3 = complete paralysis of hindquarters; 4 = very weak, unresponsive, and rapid breathing; 5 = moribund. When mice die, they were kept in the trial as score 5 for the duration of the study. Two scorers (JL and YW, each with 1.5 years of experience in EAE clinical scoring) who were blinded to the treatment groups, performed the scoring independently. The average scores from both scorers were used.

Image Analysis

Three neuroradiologists, including one who is not associated with the study (AL and YW, with 10 and 8 years of experience in interpreting images, average values from the three neuroradiologists were used for each mouse) independently analyzed the images. All radiologists were blinded to the group and clinical information to reduce potential bias, and each radiologist independently segmented the lesions to include areas that show abnormal enhancement on the 6min and 90min images compared with precontrast images. MPO-Gd images were quantified by calculating over the entire brain of each mouse: 1) lesion number, 2) lesion volume, 3) contrast-to-noise ratio (CNR), and 4) lesion activation ratio (LAR) that semiquantitatively reports in vivo MPO activity (11). Lesion volume was computed by multiplying MPO-Gd-enhancing area with slice thickness. LAR was calculated for each region of interest (ROI) as: LAR = normalized $CNR_{(90min)}/CNR_{(6min)}$. Normalized CNR = $CNR_{(6min/90min)}–CNR_{(pre)}$. CNR = $(ROI_{lesion}–ROI_{normal\ brain})/SD_{noise}$, where ROI_{lesion} is the ROI of an enhancing lesion, $ROI_{normal\ brain}$ indicates the unaffected area in the brain, and SD_{noise} is the standard deviation of noise from an ROI measuring empty space.

One engineer (GW, with 10 years of experience in postprocessing images) blinded to the group and clinical information performed 3D image renderings of 90min CNRs maps by manually segmenting the whole brain, an area outside of the mouse (noise) and an area of normal brain on the 90min post MPO-Gd image using Amira 5.3.2 (commercially available, ThermoFisher Scientific, Hillsboro, OR). In addition, the ventricles of a 3 × 3 median filtered T2 image was segmented by using a threshold that was 3 standard deviations above the mean value of normal brain tissue ROI. Voxel-by-voxel calculation of the CNR maps, with ventricle removed, compared with normal brain was performed in a Matlab R2015a (commercially available, Mathworks, Cambridge, MA) script.

Histopathologic Analysis

Six-µm fresh-frozen brain slides were examined for demyelination and MPO protein. We detected demyelination with luxol fast blue (DBS, Pleasanton, CA) according to manufacturer's instructions. We examined adjacent slides for MPO protein using anti-MPO (Ab-1, Thermo Fisher Scientific, Waltham, MA). One engineer (YI, with 10 years of experience in histology)

blinded to the group and clinical information captured images by NanoZoomer 2.0-RS (Hamamatsu Photonics, Hamamatsu, Japan). Two radiologists (MA and AL, each with 2 years of experience in interpreting images; average values were used) blinded to the group and clinical information analyzed the images using ImageJ v1.8.0 (open source,National Institutes of Health, Bethesda, MD) independently.

Isolation of Brain Inflammatory Cells and Flow Cytometry

We extracted leukocytes from the brain over a discontinuous Percoll gradient, as previously described (16). All antibodies were purchased from BD Bio-science unless otherwise indicated. We used the following antibodies: anti-CD90, 53–2.1; anti-NK1.1, PK136; anti-B220, RA3–6B2; anti-CD49b, DX5; anti-Ly-6G, IA8; anti-CD11b, M1/70; anti-Ly-6C, AL-21; and anti-F4/80, C1:A3–1 (BioLegend, San Diego, CA). We acquired data with a flow cytometer (LSR II; BD Bio-sciences, San Jose, CA) and analyzed with FlowJo V10.5.4 (commercially available, Tree Star, Ashland, OR). BP and AL (with 4 and 2 years of experience in flow cytometry; average values were used for each mouse) who were blinded to the group information and clinical scores, performed flow cytometry analysis independently. The gating strategy can be seen in Figure E1.

Statistical Analysis

Results were reported as mean ± SE of measurement. The data were tested for normality by the Shapiro-Wilk Normality test. If normality was not rejected, one-way ANOVA followed by Dunnett's multiple comparisons test (histopathologic analysis of MPO), or two-way ANOVA followed by Tukey multiple comparisons test (lesion number, lesion volume, CNR_{6min} and LAR for MRI images; flow cytometry) were used. Otherwise, we used the nonparametric Kurskal-Wallis test followed by Two-stage step-up method of Benjamini, Krieger and Yekutieli (onset of disease, clinical score on day12, histopathologic analysis of LFB). For survival analysis, Kaplan-Meier curve and log-rank test were used. $P < .05$ was considered statistically significant. When multiple samples were used for each animal, all the samples for each animal were first averaged to generate a single value before pooling the data for analysis. Prism 7 (commercially available, GraphPad Software, San Diego, CA) was used for statistical analysis.